SCANDINAVIAN JOURNAL OF
UROLOGY AND NEPHROLOGY
SUPPLEMENTUM 62

URATE METABOLISM
and
renal calcium stone disease

by
BENGT FELLSTRÖM

UPPSALA 1981
Distributed by Almqvist & Wiksell Periodical Company
Stockholm, Sweden

SCANDINAVIAN JOURNAL OF
UROLOGY AND NEPHROLOGY
SUPPLEMENTUM 62

From the Department of Internal Medicine,
University Hospital, Uppsala, Sweden

URATE METABOLISM
and
renal calcium stone disease

by
BENGT FELLSTRÖM

UPPSALA 1981
Distributed by Almqvist & Wiksell Periodical Company
Stockholm, Sweden

Doctoral thesis at the University of Uppsala, 1981.

ISSN 0300-8886

ABSTRACT

Fellström, B. 1981. Urate metabolism and renal calcium stone disease. 59 pp. Uppsala.

Both hyperuricaemia and hyperuricosuria have been considered to be common in renal calcium stone disease. Abnormalities in serum or urinary urate was not more common in 450 investigated patients than in 90 healthy controls, but when present it was often associated with a more complicated course of the disease, particularly in combination with renal acidification defects.

Hyperuricosuria was not explained by a renal tubular dysfunction of urate transport. A decreased urate clearance in proximal renal acidification dysfunction was associated with a high tubular reabsorption of filtered urate.

Sodium urate or uric acid crystals did not adsorb urinary macromolecular inhibitors of crystal growth other than in calcium oxalate solution, whereby a substantial loss of the inhibitory activity was detected.

A high intake of dietary animal protein had an unfavourable effect, as several urinary constituents potentially important for calcium stone formation were aggravated. The urinary excretion of calcium and urate increased, whereas urine pH and urinary citrate decreased. It was concluded that the protein content of the diet may be an important factor contributing to the increase in renal calcium stone disease in the industrialized world.

UNIV.
LEIDEN
BIBL.

Printed in Sweden by
Centraltryckeriet, Uppsala, 1981.

To Kristina, Anna and Stina

This dissertation is based on the following papers which are referred to by their Roman numerals:

I. Uricaemia and urinary acidification in renal calcium stone disease. B. Fellström, U. Backman, B.G. Danielson, G. Johansson, S. Ljunghall & B. Wikström. Submitted for publication.

II. Urinary excretion of urate in renal calcium stone disease and in renal tubular acidification disturbances. B. Fellström, U. Backman, B.G. Danielson, G. Johansson, S. Ljunghall & B. Wikström. Submitted for publication.

III. The inhibitory activity of human urine on calcium oxalate crystal growth - effects of sodium urate and uric acid. B. Fellström, U. Backman, B.G. Danielson, K. Holmgren, S. Ljunghall & B. Wikström. Submitted for publication.

IV. Renal handling of urate in patients with calcium stone disease. B. Fellström, U. Backman, B.G. Danielson, G. Johansson, S. Ljunghall & B. Wikström. Submitted for publication.

V. The effects of high dietary intake of protein on urinary constituents potentially important for renal stone disease. B. Fellström, B.G. Danielson, B. Karlström, H. Lithell, S. Ljunghall, B. Vessby & L. Wide. In manuscript.

CONTENTS

Abbreviations 6
Introduction 7
Urate production 7
Urate turnover rate 9
Urate elimination 10
Renal handling of urate 10
Factors affecting the renal excretion of urate 12
Urate overproduction 17
Renal impairment in hyperuricaemia 18
Uric acid urolithiasis 20
Renal calcium stone disease 21
Urate metabolism in renal calcium stone disease 24
Aims of the present studies 27
Material and methods 28
Results and discussion 30
General summary 40
Acknowledgements 42
References 44

ABBREVIATIONS

APRT	Adenine-phosphoribosyltransferase
AMP	Adenosine monophosphate
cAMP	Cyclic adenosine monophosphate
ECV	Extracellular volume
ERPF	Effective renal plasma flow
GAG	Glycosaminoglycan
GFR	Glomerular filtration rate
GMP	Guanosine monophosphate
HGPRT	Hypoxanthine-guanine-phosphoribosyltransferase
HPD	High protein diet
IMP	Inosine monophosphate
LPD	Low protein diet
PTH	Parathyroid hormone
PZA	Pyrazinamide
RTA	Renal tubular acidification defect
dRTA	Distal RTA
pRTA	Proximal RTA

INTRODUCTION

Uric acid is the end product of purine metabolism in man (Sørensen & Levinson, 1975). Some of the factors which influence the uric acid turnover rate will be reviewed as a background for the discussion of urate metabolism in relation to renal calcium stone disease.

Chemical properties of urate The purine ring structure contains four N-atoms which originate from glutamine, glycine and aspartate. Uric acid can split off two protons with pKa_1 = 5.75 and pKa_2 = 10.3 (Hitchings, 1978). The solubility of uric acid increases with pH as more urate is formed. In the presence of sodium the solubility decreases above pH 6.5 - 7.0 (Pak, Waters, Arnold, Holt, Cox & Barilla, 1977). In water a saturated solution is $\cong$ 0.4 mM at 37°C, whereas in the extracellular fluid the solubility may be even higher (Katz, 1978).

URATE PRODUCTION

De novo synthesis

The main pathways in purine metabolism are summarized in Fig. 1. The rate limiting step in the de novo synthesis is the formation of β-phosphoribosylamine (PRA) from phosphoribosylpyrophosphate (PRPP) and glutamine or ammonia (Holmes, 1978). Via nine different steps involving glycine and aspartate, the mononucleotide inosine monophosphate (IMP) is formed. Further de novo synthesis takes place via catabolism of endogenous nucleic acids from cell destruction. Dietary nucleic acids are also degraded into the mononucleotides adenosine (AMP) or guanosine (GMP) monophosphate.

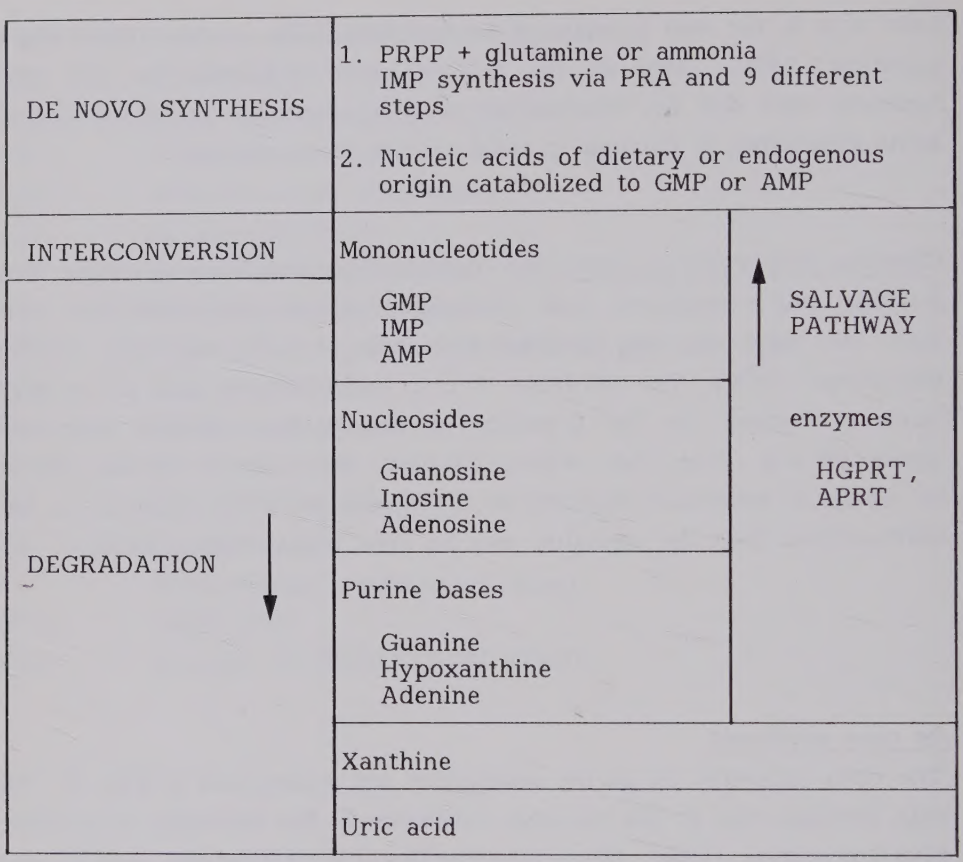

Fig. 1. Schematic presentation of purine metabolism in man.

PRPP = Phosphoribosylpyrophosphate
PRA = Phosphoribosylamine
GMP = Guanosine monophosphate
IMP = Inosine monophosphate
AMP = Adenosine monophosphate
HGPRT = Hypoxanthine-guanine-phosphoribosyltransferase
APRT = Adenine-phosphoribosyltransferase

Purine degradation

The degradation of mononucleotides is catalyzed by nucleotidases whereby phosphate is split off and the corresponding nucleosides inosine, guanosine and adenosine formed (deVries, 1980). The formation of the purine bases guanine and hypoxanthine are catalyzed by the action of nucleoside phosphorylases on GMP and IMP, respectively. Hypoxanthine is further converted to uric acid via xanthine. Both these steps are catalyzed by xanthine oxidase. The degradation of AMP proceeds via adenosine and inosine.

Purine salvage pathway

The purine bases can be reconverted to the mononucleotides by the action of the salvage enzymes hypoxanthine-guanine-phosphoribosyltransferase (HGPRT) and adenine-phosphoribosyltransferase (APRT). HGPRT-deficiency (Lesch-Nyhan syndrome) is characterized by neurologic disorders, hyperuricaemia, gout and uric acid lithiasis. In APRT--deficiency adenine is converted to 2,8-dihydroxyadenine, which has an extremely low solubility and may precipitate and cause renal damage and urolithiasis (Simmonds, van Acker, Dillon, Barratt, Potter, Sahota & Cameron, 1981).

URATE TURNOVER RATE

The fast miscible urate pool has been estimated to be about 9 mmol (Lang, Greger, Oberleithner, Griss, Lang, Pastner, Dittrich & Deetjen, 1980). The pool can be calculated from the changes in the serum level (ΔS) following the change in urate excretion (ΔUV) caused by the action of drugs affecting the excretion rate.

$$\text{Pool} = S \cdot \frac{\Delta UV}{\Delta S} \qquad (1)$$

The pool size can be assessed either through the use of uricosuric drugs such as probenecid (Lang et al., 1980), benziodarone (Lemieux,

Ninay, Gougoux & Michaud, 1973) or drugs inhibiting the excretion, such as pyrazinamide (PZA) (Steele, 1973). The reported fast miscible pool size corresponded to a distribution volume equal to about 70 % of the extracellular volume (Lang et al., 1980).

URATE ELIMINATION

In man, who lacks uricase, except from what exists in colonic bacteria, uric acid is the end product of purine metabolism. Approximately 2/3 of the urate lends itself to renal elimination, and 1/3 to intestinal elimination (Sørensen & Levinson, 1975). In the intestine uricolysis is triggered by the action of uricase in colonic bacteria, whereby urate is converted to allantoine. The intestinal fraction of uric acid elimination may double in chronic renal insufficiency, which may compensate a diminished renal elimination and partly prevent urate retention (Sørensen & Levinson, 1975).

RENAL HANDLING OF URATE

In man urate is thought to be 5 - 10 % protein bound in serum (Steele, 1978) leaving about 90 - 95 % for unrestricted glomerular filtration. The filtered urate is almost completely reabsorbed (98 - 99 %) in the early proximal tubules (Fig. 2). Tubular secretion of urate is presumably of great importance for the urinary excretion. The secretion rate is not known for certain but has been estimated to be about 50 % of the filtered load (Sørenson & Levinson, 1976b; Steele, 1978; Boss & Seegmiller, 1979). A postsecretory reabsorption of urate would seem to occur in the proximal tubules (Diamond & Paulino, 1973), and transports an estimated 4/5 of the secreted urate. The final excretion is roughly 6 - 10 % of the glomerularly filtered urate.

The urinary excretion of urate increases as a potential function of the serum level in the individual mainly as a consequence of the substrate regulated tubular secretion (Rieselbach, Sørensen, Shelp &

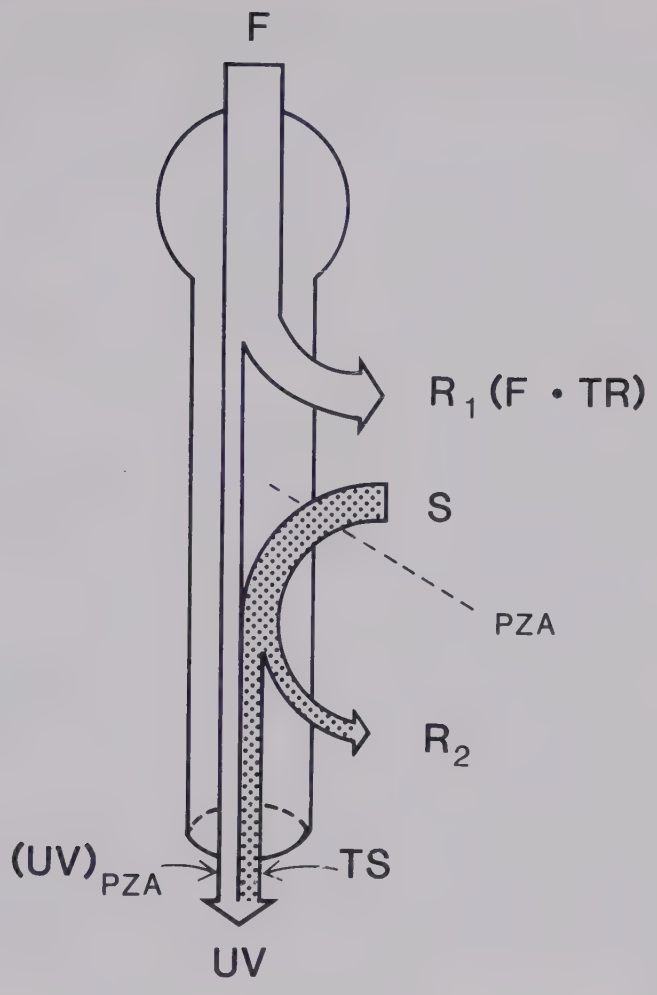

Fig. 2. Schematic model of urate transport in the nephron and the site of action of pyrazinamide (PZA). F = glomerular filtration, R_1 = tubular reabsorption of filtered urate, TR = fractional reabsorption of filtered urate, S = tubular secretion of urate, R_2 = tubular reabsorption of secreted urate, TS = net secretion rate, UV = excretion of urate, $(UV)_{PZA}$ = excretion of urate during PZA suppression of secretion. The dotted area represents urate affected by PZA.

Steele, 1970). In a group of individuals, on the other hand, no correlation could be found between serum and urinary urate (Lang et al., 1980). The renal handling can be partly studied by means of PZA suppression of urate secretion, which may yield information about the tubular reabsorption of filtered urate and the net secretion rate (Steele, 1978).

FACTORS AFFECTING THE RENAL EXCRETION OF URATE

Extracellular volume (ECV)

One of the main determinants of the renal excretion of urate is the state of the ECV (Steele, 1969). During ECV expansion the excretion increases, whereas ECV contraction causes a diminished excretion of urate. The changes in urate excretion with ECV are all within the PZA suppressible fraction of secretion (Steele, 1969).

The effects of thiazides on urate excretion could be referred to changes in ECV. Chronic treatment with thiazides causes ECV contraction and hyperuricaemia. A concomitant volume repletion restores the normal excretion of urate (Steele & Oppenheimer, 1969).

Hyperuricaemia in vasopressin resistant diabetes insipidus has been thought to be an effect of a negative water balance and ECV contraction (Gorden, Robertson & Seegmiller, 1971). In contrast ECV expansion due to an inappropriate secretion of antidiuretic hormone is accompanied by hypouricaemia, not seen in other states of hyponatremia (Beck, 1979).

Urine flow

Whether or not the urine flow per se influences the urate excretion has been a matter of debate. Meisel and Diamond (1976) suggested an increased excretion with urine flow. Another study (Engle & Steele, 1976) reported that much of this effect could be explained by changes in the ECV. Nor could Lang et al. (1980) find a relationship between urine flow and urate excretion.

Glomerular filtration rate (GFR)

The tubular transport of urate seems to be more important for urate excretion than GFR (Rieselbach et al., 1970; Sørensen & Levinson, 1976b; Steele, 1978). A decrease in GFR, as in chronic renal disease, does not cause a decrease in urate clearance of the same magnitude. This has been attributed to an increased secretion or a diminished reabsorption of urate per nephron with decreasing GFR (Steele & Rieselbach, 1967). When GFR decreased from 100 to 10 ml/min the net urate excretion decrement was about 50 % (Emmerson, 1978), and serum urate was doubled (Steele and Rieselbach, 1967).

Effective renal plasma flow (ERPF)

ERPF can be regarded as an estimate of the gross tubular function. Among patients with essential hypertension a raised serum urate level and a low renal clearance of urate were demonstrated in the individuals with low renal blood flow but unaffected GFR (Messerli, Frohlich, Dreslinski, Suarez & Aristimuno, 1980). The authors concluded that hyperuricaemia in essential hypertension could be considered to reflect an early renal vascular involvement. During infusion of angiotensin and norepinephrine a 20 % reduction in GFR was observed, whereas ERPF assessed by paraaminohippurate (PAH) clearance decreased by about 50 % (Ferris & Gorden, 1968). Simultaneously, the ratio of urate/inulin clearance declined by 45 %, which was interpreted as signifying that urate clearance was more dependent on ERPF than GFR. Whether or not PAH secretion per se was affected, and thereby the assessment of ERPF, was not discussed.

Renal acidification and urine pH

Patients with hyperuricaemia and gout have been shown to have a low urine pH with almost no diurnal variations (Gutman & Yü, 1965; Metcalfe-Gibson, McCallum, Morrison & Wrong, 1965; Pak Poy, 1965). This probably contributes to their propensity to uric acid urolithiasis. Gutman and Yü (1965) also reported a low excretion of ammonium ions

in gout and suggested a specific renal defect in the metabolism of glutamine, from being a substrate for ammonium production towards de novo synthesis of purines and eventually uric acid (Gutman & Yü, 1963). Other studies have confirmed the constantly low urine pH in gout, but not a low ammonium excretion in relation to urine pH (Barzel, Sperling, Frank & de Vries, 1964). In other reports, a lower ammonium excretion among gouty patients was attributed to a slight but general renal impairment in gout (Metcalfe-Gibson, et al., 1965; Pollack & Mattenheimer, 1965) since the renal glutaminase activity was not deficient (Pollack & Mattenheimer, 1965).

The renal excretion of urate does not seem to be enhanced by alkalinization of the urine after administration of sodium bicarbonate or acetazolamide (Ayvazian & Ayvazian, 1961; Steele, Manuel & Boner, 1975). This could have been expected as the dissociation of the acid would increase from alkalinization in the pH range of normal urine. An increased fraction of urate/uric acid would cause a diminished passive back diffusion unless the distal tubules had a low permeability for uric acid. On the other hand, during an ammonium chloride load the urate excretion was found to decrease (Gibson, Hannan, Hatfield, Simmonds, Cameron, Potter & Crute, 1977), but this was interpreted as an effect on renal production of urate.

Studies performed on renal tubular microvillus vesicles indicate an augmented transport of urate into the vesicles from a positive H^+-gradient across the membrane (Blomstedt & Aronsson, 1980), which perhaps could stimulate reabsorption of urate in an acid tubular lumen, in accordance with the rules for transport of weak acids. These results are not, however, directly applicable to proximal renal acidification defects, where a defective reabsorption of HCO_3^- presumably exists (Morris, Sebastian & MacSherry, 1972). One assumes that the pH would be high due to a HCO_3^- concentration higher than normal. During treatment with carbanhydrase (CA) inhibitors (acetazolamide or benzolamide), which creates a model for pRTA (Sohtell, 1979) it was demonstrated, however, that pH in the early proximal tubule was lower than the case prior to treatment (Vieira & Malnic, 1968; Lang, Quehenberger, Greger & Oberleithner, 1978; Karlmark, 1980). The lower pH was presumably

caused by an effect on the CA located on the luminal side of the tubular brush border membrane rather than intracellularly.

From these theoretical and model considerations one would anticipate co-existence of a defective reabsorption of HCO_3^- and an enhanced reabsorption of urate in the early proximal tubule in pRTA.

Drugs

Several drugs influence the urinary excretion of urate.

Some uricosuric drugs, which may be used in the treatment of hyperuricaemia, are sulphinpyrazone, benziodarone and probenecid (Lemieux et al., 1973; Sørensen & Levinson, 1976a; Emmerson, 1978). These are thought to interfere with the tubular reabsorption of urate, thereby increase the excretion. Other drugs are claimed to have a paradoxical dose dependent biphasic action. Salicylate, phenylbutazone and probenecid all have a dose related effect on the urinary excretion of urate (Yü & Gutman, 1955). In a low dose these drugs are thought to interfere with the tubular secretion of urate, causing a diminution of the excretion. A high dose was thought to inhibit the tubular reabsorption of urate and thereby enhance the excretion. Pyrazinamide also has a biphasic effect, but the interval between inhibition of secretion of urate and inhibition of reabsorption is wide (Weiner & Fanelli, 1975), which makes this drug more reliable than the others for studying suppression of secretion.

Diuretics may have variable effects depending on the state of the ECV. An acute injection of chlorothiazides caused an augmented urate clearance (Steele & Oppenheimer, 1969), whereas a chronic use of thiazides resulted in ECV contraction and hyperuricaemia (Manuel & Steele, 1974). The effect on the urinary excretion of urate of the chronic use of thiazides seems to be a convergence towards the normal value for the population, possibly due to a uniform state of ECV contraction in the treated patients (Ljunghall, Backman, Danielson, Fellström, Johansson, Odlind & Wikström, 1981). The changes in urate excretion due to thiazides seem to be within the PZA suppressible fraction of urate excretion (Manuel & Steele, 1974). The evidence for a connection with ECV was

obtained when it was found that an ECV restoration with saline also corrected the induced hyperuricaemia (Steele & Oppenheimer, 1969).

Metabolites and toxins

The renal excretion of urate is influenced by several metabolites and toxins. An overproduction of lactate is thought to be the cause of hyperuricaemia in alcoholism, acting through tubular competition for secretion in the kidney (Emmerson, 1978). Ingestion of sodium lactate has also been shown to cause a marked decrease in urate excretion. Reduction in urate clearance during violent exercise is probably a combined effect of a reduced renal plasma flow and an increased lactate production. Lactate is also thought to contribute to hyperuricaemia in hypertension (Cannon, Stason, Demartini, Sommers & Laragh, 1966).

A high serum concentration of ketone bodies is believed to cause hyperuricaemia by the same mechanism as lactate. Hyperuricaemia in diabetic ketoacidosis was corrected subsequent to the treatment of the ketoacidosis (Padova & Bendersky, 1962). The infusion of β-hydroxy-butyrate and sodium acetoacetate produced a striking reduction in urate clearance (Goldfinger, Klinenberg & Seegmiller, 1965). The accumulation of ketone bodies during starvation probably effectively contributes to the development of hyperuricaemia (Emmerson, 1978).

In Wilson's disease hypouricaemia was reported as a common finding and attributed to copper induced renal lesion (Wilson & Goldstein, 1973). These patients had a high urate clearance and normouricaemia was restored after treatment with penicillamine. Most of the excreted urate was PZA suppressible, indicating a lesion affecting the postsecretory reabsorption (Diamond & Paolino, 1973).

Patients suffering from acute cadmium nephropathy have been reported (Emmerson, 1967) to present what is described as a Fanconi syndrome without a phosphate leak but with high urate clearance and hypouricaemia. In contrast, lead intoxication causes a diminished urate clearance and hyperuricaemia (Emmerson, 1978). The possible role of increased lead body stores as a cause of nephropathy in gout was recently suggested (Batuman, Maesaka, Haddad, Tepper, Landy & Wedeen, 1981).

URATE OVERPRODUCTION

Hyperuricaemia has been described in several systemic diseases. The causes of hyperuricaemia can be divided into overproduction or renal underexcretion of urate. Hyperuricaemia due to overproduction of urate is usually accompanied by hyperuricosuria, in contrast to hyperuricaemia caused by renal retention. Some of the mechanisms for hyperuricaemia due to renal underexcretion have already been discussed. The causes of overproduction will be briefly reviewed.

Enzymatic defects

In Lesch-Nyhan disease, which is rare, a virtually complete HGPRT-deficiency is present and followed by neurologic disorders. The de novo synthesis of uric acid is accelerated, causing hyperuricaemia, gout and uric acid lithiasis (Arnold, 1978; Boss and Seegmiller, 1979; deVries, 1980). The partial HGPRT-deficiency is estimated to be responsible for about 1 - 2 % of gouty purine overproducers.

Glycogen storage disease type I (glucose-6-phosphatase deficiency) is accompanied by hyperuricaemia and gout, but also raised levels of triglycerides and fatty acids (Emmerson, 1978; deVries, 1980). The raised purine biosynthesis is attributed to an increased PRPP activity, which is an important enzyme in several steps of the de novo synthesis of urate (Holmes, 1978). Hyperuricaemia in this disease is, however, also caused by renal retention due to increased concentrations of both lactate and ketone bodies.

Dietary factors

A high dietary intake of purines including nucleic acids (RNA, DNA) and mononucleotides (AMP, GMP), contained to a high degree in meat, may give rise to hyperuricaemia (Zöllner, 1976). The meat content of hypoxanthine seems to be less important (Spann, Gröbner & Zöllner, 1979). The intestinal absorption of purines is almost complete regarding mononucleotides, whereas ribonucleotides are absorbed to about 50 %

(Zöllner, 1976). The ingestion of purine free proteins or amino acids does not cause hyperuricaemia but a striking hyperuricosuria. This effect is partly due to de novo synthesis of purines but also attributed to an increased clearance of urate with hypouricaemia (Löffler, Gröbner & Zöllner, 1979; Matzkies, Berg & Mädl, 1979).

Cellular destruction

The catabolism of nucleic acids caused by cellular destruction contributes to uric acid production, which becomes a problem during treatment of malignant diseases with cytostatic agents. The acute hyperuricemic renal failure (Kjellstrand, Campbell, Hartitzsch & Buselmeier, 1974) is caused by tubular obstruction of uric acid crystals, reflecting a rapid and vast overproduction of uric acid.

RENAL IMPAIRMENT IN HYPERURICAEMIA

Acute hyperuricaemic renal failure

The most dramatic example of renal impairment due to hyperuricaemia is the acute renal failure following urate overproduction in the treatment of malignant diseases (Boss & Seegmiller, 1979). This consequence of the treatment of leukemia was found in 6/57 patients (Talbott, 1978). The cause is most probably an intratubular obstruction by uric acid crystals to which dehydration and an acid urine may contribute. This type of renal insufficiency was further characterized by unusually high levels of serum phosphate (Kjellstrand et al., 1974).

Urate nephropathy

Renal functions and morphology have been extensively studied in gout. Intrarenal crystal deposition was present at autopsy in about 90 % of gouty subjects (Talbott & Terplan, 1960; Barlow & Beilin, 1968). Two forms of crystals were found. Amorphous uric acid crystals were deposited as microcalculi within the lumina of the collecting ducts in the

medulla, while the needle-like crystals of monosodium urate were found in the medullary interstitial tissues. The larger deposits of monosodium urate often contained acid mucopolysaccharides and occasionally calcium salts. The medullary interstitial deposits were often surrounded by an aseptic inflammatory reaction including multinucleated giant-cells but rarely amyloid (Talbott, 1978). The localization of the urate deposits probably depends on the combined effect of high concentration of urate and sodium (countercurrent effect), and the deposition is thought to be more likely to occur in an alkaline environment (Klinenberg, Kippen & Bluestone, 1975).

The most common structural changes in the gouty kidney were glomerular sclerosis, tubular atrophy and vascular lesions (Gonick, Rubini, Gleason & Sommers, 1965; Barlow & Beilin, 1968). Tubular dilatation was present due to microcrystal obstruction. Tubular atrophy was the most striking finding in non-diabetic gouty patients and twice as common as in control subjects, even considering that its prevalence increased with age (Barlow & Beilin, 1968). Because of the close association between gout and hypertension (Cannon et al., 1966; Gonick et al., 1965; Messerli et al., 1980) the cause of renal vascular sclerosis in the gouty kidney is not apparent. It was demonstrated, however, that in 21/23 gouty patients vascular sclerosis was present independent of hyper- or normotension, whereas in the non-gouty patients vascular sclerosis was more common if the patients were hypertensive (Barlow & Beilin, 1968). Conversely, hyperuricaemia has been suggested to be an early indication of renal vascular involvement in hypertension (Messerli et al., 1980).

The renal function thought to be first affected in gout is the ability to concentrate the urine (Barlow & Beilin, 1968). A defective concentrating mechanism was found in 40 % of patients with gout compared with 25 % of asymptomatic hyperuricaemic patients (Klinenberg, Dornfield & Gonick, 1967). One or more functional defects were found in 63 % in asymptomatic hyperuricaemia and in 83 % in gout (Klinenberg et al., 1967). In another study 4/14 hyperuricaemic patients proved to have a slightly lowered inulin clearance but in particular a low renal plasma flow as judged by PAH-clearance (Colton, Ward, Maher &

Ferguson, 1966). Furthermore, it was recently reported, (Batuman et al., 1981) that the body stores of lead were twice as high in gouty patients with azothemia as in those with a normal GFR. The possible role of lead in gouty nephropathy was proposed.

An irreversible renal involvement has been claimed to be the cause of death in 10 % of gouty patients (Talbott & Terplan, 1960). A prospective study of gout and hyperuricaemia without renal or cardiovascular disease, hypertension or diabetes mellitus disclosed that during an eight year follow-up two per cent developed azothemia both in the patient group and among the normouricaemic controls (Fessel, 1979). The risk of developing cardiovascular disease or diabetes mellitus was, however, increased in the hyperuricaemic group (Fessel, Siegelaub & Johnson, 1973).

In summary there is an apparent overrepresentation of hypertension with renal involvement in hyperuricaemia and gout. Patients with an isolated hyperuricaemia without hypertension, diabetes mellitus or signs of renal involvement do not seem to run an increased risk of developing azothemia during an eight year period. It is possible, however, that a more elaborate measurement of tubular functions would have disclosed a renal involvement.

URIC ACID UROLITHIASIS

The relation between disturbances of urate metabolism and uric acid nephrolithiasis is well established (Yü & Gutman, 1967). A principal cause may be an overproduction of urate leading to a high urinary excretion and a concomitantly increasing risk of forming uric acid stones. Normouricaemic patients may also form uric acid stones, particularly if the urine pH is low. In gout the urine pH is constantly low, which increases the propensity for formation of uric acid stones (Gutman & Yü, 1965; Metcalfe-Gibson et al., 1965; Pak Poy, 1965). A hereditary defect in tubular reabsorption of urate has also been described as leading to hypouricaemia, hyperuricosuria and uric acid stone formation (Frank, Many & Sperling, 1979; Fujiwara, Takamitsu, Ueda, Orita & Abe, 1980).

In our experience uric acid stones are rare and represent less than two per cent of the analysed stones (Backman, Danielson, Fellström, Johansson, Ljunghall & Wikström, 1980b), whereas they comprise a larger proportion of the stone spectrum in other parts of the world (deVries, Weinberger & Frank, 1973; Cifuentes-Delatte, Rapado, Abehsera, Traba & Cortes, 1973).

RENAL CALCIUM STONE DISEASE

The incidence of urolithiasis has demonstrably increased during the 20th century (Andersen, 1969; Ljunghall, 1977). The number of stone discharges appeared to be related to socio-economic factors, and changed parallel to fluctuations in the economy of the country, which evidently influences the dietary habits and the incidence of stone disease, with a 1 - 2 year lag (Robertson, Peacock, Heyburn, Speed & Hanes, 1978b).

Dietary factors

The dietary habits probably have a great impact on stone disease. Since a high dietary intake of dairy products causes hypercalciuria stone patients have often been recommended to curtail their intake of these foods. However, such restrictions should be moderated as intraintestinal calcium participates in the complex binding of oxalate, and thereby lowers the fractional absorption of oxalate (Smith, van den Berg & Wilson, 1978; Anderson, 1978). The intestinal absorption of oxalate is normally thought to be less than 15 % (Williams, 1976), but may rise when the intestinal calcium available for complexing is decreased as during low calcium intake or steatorrhoea (Williams, 1976; Andersson, 1978).

The urinary excretion of calcium may be influenced both by calcium content of the diet and by dietary factors affecting the absorption and excretion rates. Refined carbohydrates have been shown to induce hypercalciuria, possibly due to a renal loss or a stimulated calcium absorption (Lemann, Piering & Lennon, 1969; Thom, Morris, Bishop &

Blacklock, 1978). Dietary protein also causes hypercalciuria which in one study was thought to be the result of an increased intestinal absorption (McCance, Widdowson & Lehmann, 1942). Later balance studies have shown an unaltered intestinal absorption but a decreased renal tubular reabsorption of calcium (Johnson, Alcantara & Linksweiler, 1970; Allen, Bartlett & Block, 1979).

Dietary animal protein also increased the urinary output of oxalate, probably because oxalate is the end product of the catabolism of several amino acids contained to a high degree in meat (Robertson, Heyburn, Peacock, Hanes & Swaminathan, 1979a; Butz, Hoffman, Kohlbecker & Brosig, 1981).

Robertson estimated that the stone forming risk increased by 250 % after the addition of 35 g animal protein to a regular diet (Robertson et al., 1979a). Vegetarians had the lowest risk, based on urinary constituents, of forming calcium containing stones. In a recent survey, the incidence and prevalence of stone disease among vegetarians was much lower than in the pre-vegetarian period (Robertson, 1981).

Physico-chemical aspects

In urolithiasis calcium containing stones are the most common, including both calcium oxalate, calcium phosphate and a mixture of the two salts. One factor determining whether a crystal or a stone will be formed is the balance between the supersaturation of the urine and the inhibitory activity of crystal formation, growth and aggregation (Robertson, Peacock & Nordin, 1971; Robertson, Peacock, Marshall, Marshall & Nordin, 1976b; Fleisch, 1978). The degree of supersaturation is determined by the ionic activity product of the salt, which can be either under- or supersaturated. The lower limit of metastability is the solubility product, below which the salt is undersaturated. The upper limit is the formation product, above which the salt may precipitate spontaneously. In the metastable region a crystallization and growth may occur if the process is started for example by the addition of crystals.

The degree of supersaturation of calcium oxalate in the urine can hardly exceed the formation product by increasing the calcium concentra-

tion (Robertson, Peacock & Nordin, 1968), whereas small changes in the oxalate concentration exert a greater influence. For this reason, it was proposed that even a moderate hyperoxaluria is more important than hypercalciuria because of the relative supersaturation of calcium oxalate in the urine (Robertson & Peacock, 1980).

Most urines are supersaturated without stones being formed. The reason is the existence of inhibitors in the urine. There are basically two modes of inhibitory action. One is a mechanism whereby urinary constituents form soluble complexes with calcium (citrate, sulphate) or oxalate (magnesium), and cause a decrease in the ion activity product. The other mechanism is the inhibition of crystal formation, growth and aggregation, in which both low (citrate, pyrophosphate) and high molecular weight (glycosaminoglycans (GAG's), ribonucleotides) urinary constituents are involved.

Urinary GAG's are apparently the principal macromolecular inhibitors of crystal growth and aggregation (Robertson, Peacock & Nordin, 1973; Bowyer, Brockis & McCulloch, 1979). The initial precipitation of calcium oxalate is, however, also inhibited by several kinds of GAG (Pak, Holt & Zerwekh, 1979). Brockis could recover chondroitin sulphates from a calcium oxalate crystal surface but no other type of GAG was identified. GAG's are probably most important as inhibitors of crystal aggregation (Robertson, personal communication), and a disabled GAG activity may cause aggregation of calcium oxalate crystals.

Other macromolecular inhibitors of crystal growth have also been identified in the urine (Schrier, Lee, Rubin, Werness & Smith, 1978) and thought to have a RNA-like structure. The inhibitory activity was estimated to be about 50 % of the total activity, according to a crystal growth assay.

The urinary excretion of calcium is a well established risk factor of calcium stone disease (Ljunghall, 1977) and hypercalciuria is usually caused by an intestinal hyperabsorption of calcium (Pak, Ohata, Lawrence & Snyder, 1974; Ljunghall, Backman, Danielson, Johansson, Jung, Källsen, Wibell & Wålinder, 1977). A so-called renal leak of calcium, where the primary defect is located in the renal tubules, has been found in about 10 % of the patients with hypercalciuria (Pak et

al., 1974). Hypercalciuria due to a primary bone resorption would seem to be rare in normocalcaemic patients (Ljunghall, Backman, Danielson, Fellström, Johansson & Wikström, 1980).

Urinary magnesium has been shown to inhibit calcium phosphate crystallization (Fleisch, 1978). Magnesium metabolism was found to be normal in renal stone patients (Johansson, 1979), who nevertheless may benefit from treatment with magnesium hydroxide (Johansson, Backman, Danielson, Fellström, Ljunghall & Wikström, 1980) since they had an abnormal urinary calcium/magnesium ratio, due to a high calcium excretion.

In our experience renal tubular acidification defects are common in calcium stone disease (Backman, Danielson, Johansson, Ljunghall & Wikström, 1980a). Predominantly incomplete forms of proximal or distal renal tubular acidification defects were found in 20 % of the patients. They had also other signs of renal tubular dysfunctions such as an increased frequency of tubular proteinuria and also a low urinary excretion of citrate (Wikström, Backman, Danielson, Fellström, Johansson & Ljunghall, 1981). Their clinical picture was characterized by an early onset of the stone disease, which was rather complicated in terms of stone operations.

URATE METABOLISM IN RENAL CALCIUM STONE DISEASE

There are several implications for a relationship between abnormalities in urate metabolism and calcium stone disease. In 25 % of uric acid stones calcium was found in varying proportions (Gutman & Yü, 1968), and urate was included in 4 % of calcium stones (Rapado, Castrillo, Diaz-Curiel, Traba, Santos & Cifuentes-Delatte, 1979). Hyperuricaemia has further been claimed to be common in calcium stone disease (Smith, Hunt, Cling & Boyce, 1969; Dent & Sutor, 1971), and a high urinary excretion of urate has been proposed to be a feature in calcium stone disease (Coe & Kavalach, 1974; Robertson, Peacock, Heyburn, Marshall & Clark, 1978a).

Hyperuricosuria has been attributed to a high consumption of purine rich animal protein in the industrialized world (Coe, Moran & Kavalach, 1976; Robertson et al., 1978b) and the question has been raised whether stone formers should be recommended to become vegetarians (Robertson, Peacock, Heyburn, Hanes, Rutherford, Clementson, Swaminathan & Clark, 1979b).

Dietary animal protein also increases the urinary excretion of calcium and oxalate and thereby supposedly increases the supersaturation of calcium oxalate. It is possible that hyperuricosuria is only a coincidence with no pathogenetic significance for calcium stone disease but some modes of causal relationship have been proposed.

Various mechanisms have been suggested, whereby urinary urate may facilitate the precipitation, crystal growth and aggregation of calcium oxalate. One mechanism is that monosodium urate crystals may induce an epitaxial growth of calcium oxalate (Coe, Lawton, Goldstein & Tembe, 1975; Pak & Arnold, 1975). This theory is supported by crystallographic analysis, which demonstrated a similarity between the crystal structure of monosodium urate and calcium oxalate (Lonsdale, 1968). The existence of stones containing a mixture of urate and calcium oxalate is further evidence for this theory (Gutman & Yü, 1968; Rapado et al., 1979).

Another possible mechanism is that microcrystals of sodium urate bind urinary GAG's (Robertson, Knowles & Peacock, 1976a; Pak, Barilla, Holt, Brinkley, Tolentino & Zerwekh, 1978; Pak, Holt & Zerwekh, 1979) and cause an attenuated inhibitory activity, as these urinary macromolecules are potent inhibitors of calcium oxalate crystal aggregation (Robertson et al., 1973). Robertson showed an inverse relationship between the acid mucopolysaccharide inhibitory activity of calcium oxalate aggregation and the urate concentration in urine (Robertson et al., 1976a). Pak studied the interaction between several urate salts and pure fractions of GAG's, such as heparin and chondroitin sulphates. The presence of both heparin and chondroitin sulphates demonstrably increased the formation product ratio (FPR) of calcium oxalate. This effect did not occur if heparin was previously incubated with sodium urate crystals (Pak et al., 1979), indicating its adsorption onto the

crystals. The kinetics of this adsorption phenomenon were studied in relation to calcium and proved to increase with the concentration of calcium (Finlayson & duBois, 1978). Sodium urate crystals did not adsorb chondroitin sulphates, however.

The relevance of heparin as a model for urinary GAG's must be questioned as heparin does not exist other than in minute amounts in the human urine (Varadi, Cifonelli & Dorfman, 1967; Wessler, 1971). It is not clear whether it exists extracellularly at all in man (Comper & Laurent, 1978). The augmented adsorption of heparin on sodium urate crystals in the presence of calcium may be explained by a 100 times higher affinity of the polyanionic GAG's to divalent compared with monovalent cations (Comper & Laurent, 1978).

Since urate metabolism seems to be of relevance for renal calcium stone formation a series of investigations was designed.

AIMS OF THE PRESENT STUDIES

The aims of the present studies were to

- establish the extent of hyperuricaemia and hyperuricosuria and their clinical importance in renal calcium stone disease.

- investigate the conditions under which urate may interfere with naturally occurring urinary macromolecular inhibitors of calcium oxalate crystal growth.

- evaluate the renal handling of urate in calcium stone patients and in particular study whether hyperuricosuria was the result of a defect in the renal handling of urate.

- investigate the metabolic effects of dietary animal protein with special reference to factors of potential importance for renal stone formation.

MATERIAL AND METHODS

Patients

In the first two studies (I, II) the patients were consecutive cases at our outpatient stone clinic (Backman, Danielson, Johansson, Ljunghall & Wikström, 1979) and had undergone our metabolic evaluation. The studies comprised altogether 487 calcium stone formers, 359 males and 128 females (I), and 467 patients, 350 males and 117 females (II) referred to our clinic because of recurrent stone disease. All had a normal GFR. No patients had primary hyperparathyroidism, cystinuria or were taking drugs which may affect serum or urinary urate. Healthy stone-free controls (I, II), free of medication, were recruited from a health survey (Danielson, Johansson & Ljunghall, 1979).

For the investigation of renal handling of urate (IV) 28 stone patients were randomly selected. Twelve were hyperuricosuric (> 4.5 mmol/24 h in males and > 4.0 mmol/24 h in females), eight had a proximal and eight a distal renal tubular acidification defect (Backman et al., 1980a). Twelve patients were on stone prophylactic medication, which was suspended one week prior to the study. Twelve healthy members of the staff served as controls.

Another eight calcium stone formers were randomly selected for the investigation of the metabolic effects of dietary animal protein (V). The patients had undergone our outpatient metabolic evaluation prior to the study. Four patients had hypercalciuria (> 7.5 mmol/24 h), one hyperuricosuria (6.3 mmol/24 h) and one an incomplete proximal renal tubular acidification defect (ipRTA). Stone prophylactic medication was suspended two weeks before admission.

Methods

Our metabolic evaluation of stone patients included analysis of electrolytes and urate in serum and urine, urinary citrate, urine culture and a qualitative test for cystinuria. The urinary acidification capacity was evaluated from a short-term ammonium chloride load (Backman et al., 1980a), which allows diagnosis of both incomplete and complete forms of proximal and distal renal tubular acidification defects.

Stone analysis was performed by infrared spectrophotometry. A stone episode was defined as the spontaneous passage, surgical removal or radiographic visualisation of a stone, and traced from old records, case histories and previous radiograms. Stone operations included nephro-, pyelo-, and uretero-lithotomy together with endoscopic stone removal. The stone episode rate was the number of stone episodes, and the stone operation rate the number of stone operations in 100 patient years (I, II).

Hyperuricosuria was arbitrarily defined as > 4.5 mmol/24 h in males and > 4.0 mmol/24 h in females. Hyperuricaemia was defined as > 400 μmol/l in males and > 300 μmol/l in females. These wide limits corresponded approximately to mean + 1 S.D. in healthy controls and were used in order to recruit a fairly large number of patients for the analysis of the clinical characteristics of hyperuricosuria and hyperuricaemia (I, II). The stone history of these patients was compared with normouricosuric and normouricaemic patients, who were matched on the basis of age and sex (I, II).

The inhibitory activity of human urine on calcium oxalate crystal growth was examined in a seeded crystal growth system (III). Freshly voided urine from five healthy subjects was pooled, millipore-filtered and separated with ultrafiltration using a filter with a cut-off at 10,000 Dalton (YM-10, Amicon). The inhibitory activity of the ultrafiltrable and the macromolecular fractions of the urine was examined through addition to the crystal growth system. The degree of retardation of the crystal growth rate was an estimate of the inhibitory activity. The crystal growth kinetics were monitored by following the disappearance of ^{14}C-oxalate from solution. The effects on the inhibitory activity of a two hour preincubation of the urinary fractions with calcium oxalate, sodium urate and uric acid crystals were also studied.

The renal handling of urate was investigated through pyrazinamide (PZA) suppression of the renal tubular secretion of urate (IV). The study was made after a 12 hour fast. The subjects drank water to sustain the diuresis. After two initial 30 minute urine collections 3 g PZA were given orally. After 90 minutes another three 30 minute urine collections were made. Urate and creatinine were analysed in serum and in urine.

The filtered load, tubular reabsorption of filtered urate and the net secretion of urate could be calculated from the pre- and post-PZA values of urate and creatinine in serum and urine.

The metabolic effects of dietary animal protein were studied by feeding the patients a low protein (LPD) and a high protein (HPD) diet (V). The patients spent four weeks in a metabolic ward, two weeks on each diet. The LPD contained 12 energy per cent protein and 48 % carbohydrates, and the HPD 29 % protein and 31 % carbohydrates. By the end of each period two consecutive 24 hour urine collections were made together with a fasting 2 hour urine collection in the morning.

Laboratory methods

Calcium and magnesium were analysed by atomic absorption spectrophotometry, urate by a uricase method (Kageyama, 1971), citrate with a citrate lyase method (Welshman & McCambridge, 1973), oxalate by a colorimetric procedure (Hodgkinson & Williams, 1972) and sulphate by ionic chromatography (Rich, 1977). Urinary cystine was determined qualitatively (Brand, Harris & Biloon, 1930), urinary hydroxyproline colorimetrically, urinary cyclic AMP (cAMP) (Wålinder, Ljunghall & Wibell, 1978) and serum PTH (Arnaud, Tsao & Littledike, 1971; Hehrmann, Nordmeyer, Wilke & Hesch, 1977) with a radioimmunoassay technique.

The thermodynamic equilibrium of the urine was calculated by employing the iterative procedure EQUIL (Finlayson, 1977). The limits of metastability for various ion pairs were adopted from Marshall & Robertson (1976).

Conventional statistical methods were applied.

RESULTS AND DISCUSSIONS

Serum and urinary urate in stone formers and controls (I, II)

Males had a higher serum urate than females ($p < 0.001$), and controls had higher levels than stone formers ($p < 0.001$). The mean values ± 1 S.D. (μmol/l) were 347 ± 56 (male controls), 308 ± 70 (male stone

formers), 262 ± 48 (female controls), and 226 ± 58 (female stone formers). Females above 50 years of age had a higher level than the younger females ($p < 0.05$).

The urinary excretion of urate (mmol/24 h) in male stone formers (3.3 ± 1.0) was not different from male controls (3.5 ± 1.1). Similarly the excretion in female stone formers (3.0 ± 0.9) did not diverge from female controls (2.9 ± 0.9). The frequency of hyperuricosuria was equal in stone formers and controls. Males had a higher excretion than females ($p < 0.001$). The decrease in the urinary excretion of urate with age in female and male controls was 0.2 mmol/24 h/10 years of age ($p < 0.01$).

Patients with a proximal renal tubular acidification defect (pRTA) had a higher serum urate (mean difference 24 μmol/l) than those with a normal acidification of the urine ($p < 0.02$). The urinary excretion was not significantly different (mean difference -0.3 mmol/24 h) but the corresponding urate clearance (ml/min) in pRTA (7.2 ± 2.7) was lower than in normal acidification of the urine (8.5 ± 3.3) ($p < 0.02$). The relation between renal acidification and renal handling of urate will be discussed below.

In contrast to previous investigations (Smith et al., 1969; Dent & Sutor, 1971) serum urate was not increased in our patients, and in contrast to Coe and Robertson (Coe & Kavalach, 1974; Robertson et al., 1978a) urinary urate was not increased in our stone patients, compared with healthy controls. In two other reports too (Hodgkinson, 1976; Schwille, Samberger & Wach, 1976) hyperuricaemia or hyperuricosuria were not striking features of calcium stone disease. The reasons for the variable results are not clear but may be due to dietary or socio-economic differences, which may influence at least the urinary excretion of urate (Coe et al., 1976; Robertson et al., 1979a; Robertson et al., 1978b).

Clinical characteristics in relation to hyperuricaemia, hyperuricosuria and renal acidification (I, II)

Both hyperuricaemia and hyperuricosuria were associated with higher stone operation rates than normouricaemia or normouricosuria, respec-

tively ($p < 0.001$). The hyperuricaemic patients had undergone 8.9 stone operations in 100 patient years compared with 3.7 in normouricaemic patients. Patients with hyperuricaemia and RTA had the highest operation frequency (16.1), compared with hyperuricaemic patients with a normal acidification of the urine (6.5). In normouricaemia there was no difference in operation rates between patients with a normal and a defective acidification of the urine.

Similarly, patients with hyperuricosuria had a higher operation rate (8.4) than normouricosuric patients (4.1). Hyperuricosuric patients with RTA had a higher operation rate (16.1) than hyperuricosuric patients with a normal urinary acidification (6.5). In normouricosuria there was no difference between patients with a normal or a defective urinary acidification.

A high stone operation frequency in hyperuricosuric stone formers was previously reported (Coe, 1977) but the reason is not clear. It has been shown in rats that the combined induction of hyperuricosuria and hyperoxaluria caused larger stones than an isolated hyperoxaluria (Hering, Bigalke & Lutzeyer, 1979). Sodium urate microcrystals may interfere with GAG's (Pak et al., 1979) which are thought to be potent inhibitors of calcium oxalate crystal aggregation. It has also been reported that the solubility of sodium urate decreases with an increasing pH (Pak, et al., 1977). This prompts the hypothesis that hyperuricosuric patients with renal acidification defects run an increased risk of forming sodium urate deposits in the renal medulla or pyramids, where the sodium concentration is high due to countercurrent effects. Sodium urate crystals would possibly bind GAG's and leave a decreased urinary inhibition of calcium oxalate aggregation.

A similar mechanism may include hyperuricaemia in combination with renal acidification defects, and take place either inside the tubular lumen because of a high filtered load of urate, or in the medullary interstitium due to a high peritubular concentration of urate. Conglomerates of monosodium urate, acid mucopolysaccharides and calcium salts have been demonstrated in the gouty kidney (Talbott, 1978).

It has previously been shown that stone patients with renal acidification defects had been operated more frequently than others (Backman

et al., 1980a), whereas in these studies (I, II) a combination with urate disturbances seemed to be important. In addition to the hyperuricaemic, hyperuricosuric and matched control patients (I, II) the study was extended to include all patients with renal acidification defects, in whom urate values had been measured prior to prophylactic medication. Since hyperuricaemia and hyperuricosuria in some instances occurred together (I, II) the patients were now classified according to isolated abnormalities such as hyperuricaemia, hyperuricosuria, RTA or any possible combination. It was found that patients with an isolated RTA had not been operated more than patients free of all these anomalies, whereas the operation frequency was highest when a combination between RTA and hyperuricaemia, hyperuricosuria or both was present (Fig. 3 and Fig. 4). It must be pointed out that only one patient had hyperuricaemia, hyperuricosuria and RTA.

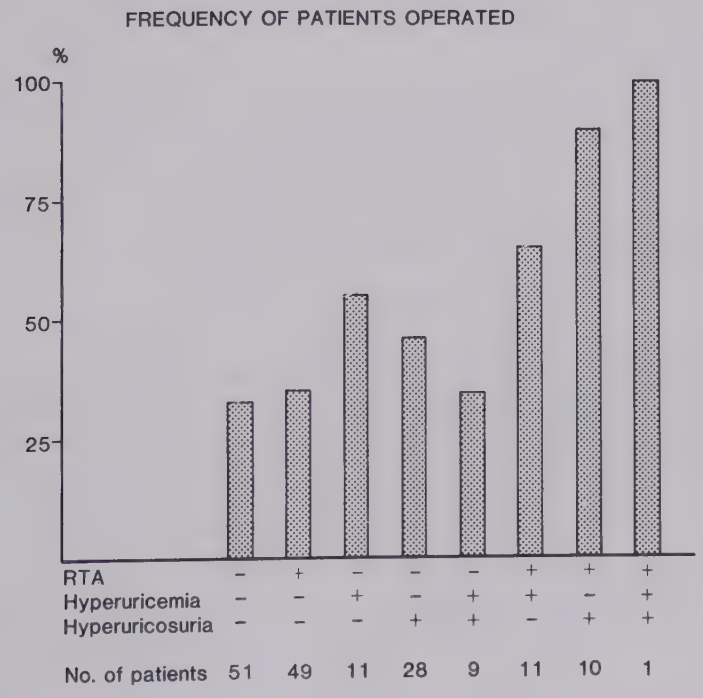

Fig. 3. Frequency of patients operated (%).

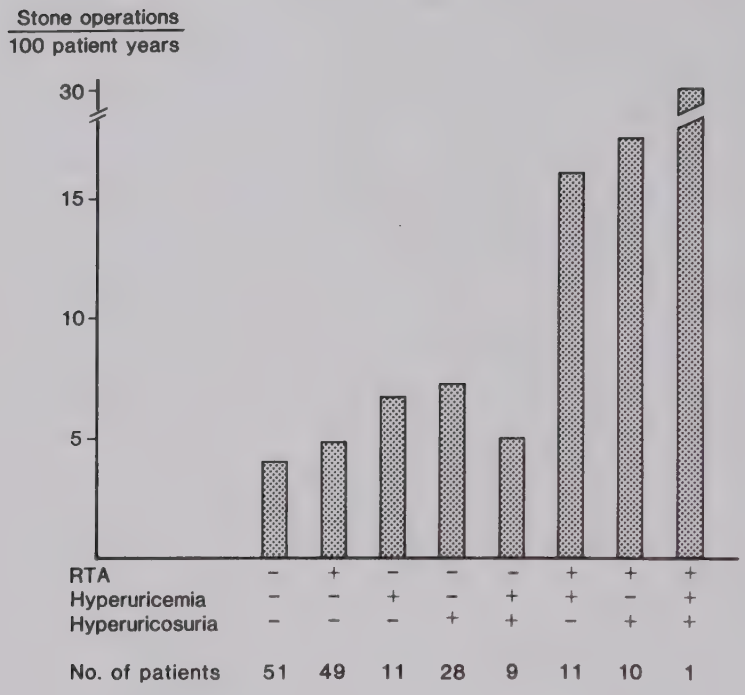

Fig. 4. Stone operations per 100 patient years.

Urinary inhibitors of calcium oxalate crystal growth (III)

Both the ultrafiltrable and the macromolecular fractions of human urine were potent inhibitors of calcium oxalate crystal growth. The ultrafiltrate was somewhat more efficient, which contradicts the results of other investigators (Schrier et al, 1978; Bowyer, et al., 1979) and may be due to the high concentration of inhibitor which was used here (9 %). As there is no linear relationship between the concentration and the activity of urinary inhibitors, the interrelation between the two fractions will not necessarily be equal to a situation with lower concentrations (Fleisch, 1978). The discrepancy may also be due to the greater potency

of macromolecules as inhibitors of aggregation than of crystal growth (Robertson, personal communication).

Preincubation of the urinary macromolecular fraction with calcium oxalate crystals caused an almost complete disappearance of the inhibitory activity in contrast to the effect on the ultrafiltrate, where the inhibitory activity was only slightly decreased by the same maneuver. These results were interpreted as indicating a more permanent adsorption of the macromolecular than of the ultrafiltrable fraction onto the crystals.

The preincubation of the urinary fractions with uric acid or sodium urate crystals only slightly curtailed the inhibitory activity, but when the incubation was performed in the presence of calcium (1.5 mM) and oxalate (0.1 mM) in the solution a substantial loss of inhibition ensued. The residual inhibitory activity of the macromolecular fraction was 45 % after incubation with uric acid crystals and 20 % with sodium urate crystals.

It has been shown in vitro that sodium urate may adsorb heparin but not chondroitin sulphates (Pak et al., 1979). The latter are, however, the major urinary GAG's (Varadi et al., 1967; Wessler, 1971). As heparin is not excreted in the human urine and probably does not exist extracellularly in man, heparin can be questioned as a model for urinary GAG's.

The urinary macromolecular fraction as separated here probably contained most of the urinary GAG's (Comper & Laurent, 1978). It being an essential property of the polyanionic GAG's that divalent cations are bound much more potently than monovalent cations, it is not surprising that in the presence of calcium the decrement in the inhibitory activity was larger. A similar dependence on calcium concentration has been reported for heparin adsorption on sodium urate crystals (Finlayson & duBois, 1978). Whether or not an epitaxial growth of calcium oxalate had occurred (Coe et al., 1975), due to structural similarities between sodium urate and calcium oxalate (Lonsdale, 1968), creating an adsorptive site for GAG's on the crystal surface, cannot be determined.

A decrement in the inhibitory activity was also seen when the incubation was made with uric acid crystals in calcium oxalate solution, although the effect was less pronounced than with sodium urate. This effect may be significant for the metabolic consequences of dietary animal protein, where a ten-fold increase was found in the supersaturation of uric acid in the urine (V).

Renal handling of urate (IV)

The oral administration of 3 g PZA caused an 85 % reduction in urate excretion due to suppression of tubular secretion (Weiner & Fanelli, 1975). The average effect on the serum level of urate was an increase by 6 μmol/l/h (Fig. 5). The interindividual variation was wide, however. With an average excretion decrement of 2.4 μmol/min the fast miscible pool could be calculated (1) to be 24 l (32 % of the body weight), which is close to a previous report (Lang et al., 1980), and slightly larger than the ECV. With an average serum urate concentration of 350 μmol/l in males, and an urinary excretion of urate 3.5 mmol/24 h (I, II), the daily turnover rate would be 40 % of the urate pool.

Judging by the PZA suppression of renal tubular secretion of urate hyperuricosuria could not be explained by abnormalities in the renal handling of urate since these patients had a tubular reabsorption of filtered urate and a net secretion of urate not different from the controls.

The patients with proximal RTA had a higher reabsorption of filtered urate, whereas their fasting excretion was equal to the controls. It is suggested that during a dietary load of urate the high reabsorption of filtered urate may contribute to the low clearance of urate based on 24 hour urine collections that was reported above (II).

Patients with pRTA have a defective reabsorption of HCO_3^-. The co-existence with an enhanced reabsorption of urate may be explained by a simultaneous low pH and a high HCO_3^- concentration in the early proximal tubule (Viera & Malnic, 1968; Lang et al., 1978). A positive H^+-gradient has been shown to stimulate the transport of urate across tubular microvillus vesicles (Blomstedt & Aronson, 1980) and may be a mechanism in pRTA.

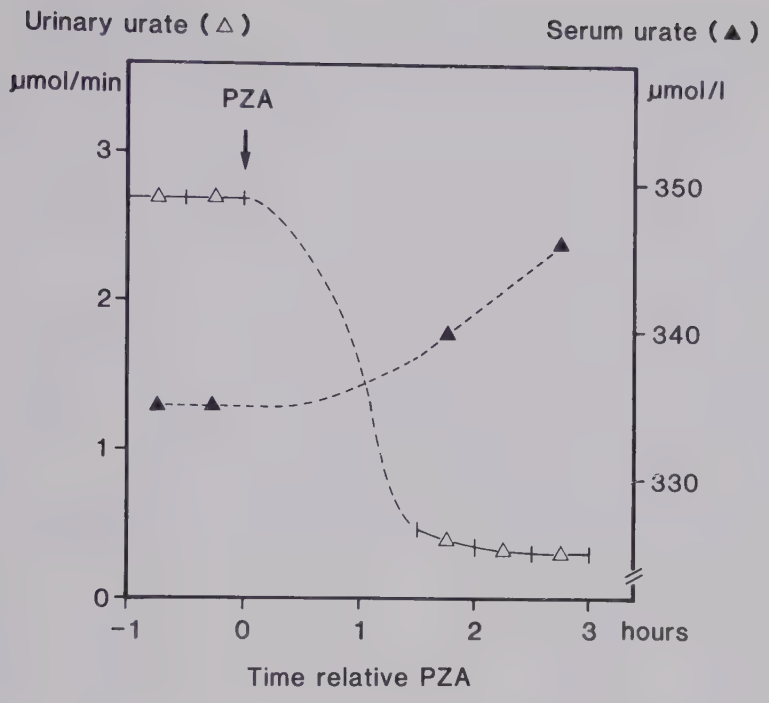

Fig. 5. The average effects of PZA on the urinary excretion of urate (Δ) and the serum urate levels (▲).

The PZA suppressible amount of urate is thought to represent a net secretion of urate and to be the principal regulator of urate excretion. It was quantitatively more important than the fraction of filtered urate which escaped reabsorption, but not correlated to the 24 hour excretion of urate.

The tubular reabsorption of filtered urate was inversely correlated to the 24 hour excretion of urate. This mechanism may be important for the regulation of urate excretion on a free diet.

The metabolic effects of a high dietary animal protein (V)

Several factors which have an adverse effect on urolithiasis were aggravated by a high intake of dietary animal protein (HPD) compared with a low protein diet (LPD). The urinary excretion of calcium increased from 5.5 to 7.4 mmol/24 h, 70 % of the increase being in the night portion. Serum calcium did not change. Both the 24 hour and the fasting 2 hour excretion of cAMP increased, whereas serum PTH remained constant. The fasting excretion of hydroxyproline increased as well.

A primary renal loss of calcium was thought to occur, probably due to a decreased tubular reabsorption of calcium. Compensatory mechanisms may involve transitory increases in PTH-secretion as reflected by cAMP. It is believed that bone buffering of the acid load due to the proteins may have caused some degree of bone resorption as reflected by the increased fasting urinary hydroxyproline.

The excretion of sodium, potassium, sulphate, titratable acid and ammonium ions also increased. The total increase in acid excretion was 0.5 mmol/g protein, similar to a previous report (Lennon, Lemann & Relman, 1962). Despite an increased buffering capacity pH decreased by 0.9 pH units. The average decrease in standard bicarbonate, within the normal range, was 1.1 mmol/l.

Urinary citrate decreased from 4.3 to 3.1 mmol/24 h ($p < 0.001$), possibly because of the increased acid load causing an enforced renal utilization of citrate (Cohen & Barac-Nieto, 1973). The average urinary oxalate was unchanged but an increase was seen in four individuals.

The calculated ionized fraction of urinary calcium increased from 40 to 49 % and the 24 hour urinary Ca^{++} increment was 1.4 mmol. The calcium sulphate complex was 1.3 mmol on HPD compared with 0.4 mmol on LPD. In contrast the calcium citrate complexes decreased by 0.5 mmol/24 h. The net increase in calcium complexes was 0.5 mmol/24 h. The ion activity product of calcium oxalate was unchanged, mainly because of the lack of increase in urinary oxalate and the slight increase in urine volumes in 7/8 patients.

The combined purine-protein load had no effect on serum urate, but the excretion of urate increased by 90 %. The uricosuric effect of dietary protein (Löffler et al., 1979) probably balanced the expected

hyperuricaemia due to the purines (Zöllner, 1976). The excretion of undissociated uric acid was 11 times higher on HPD than LPD due to both urinary urate and pH. The ion activity product of uric acid was in the supersaturated region on HPD but not on LPD and in two cases even above the formation product of uric acid. The ion activity product of sodium urate was no higher on HPD, because the anticipated increase was counteracted both by pH and the slight increase in urine volume.

The inhibitory activity of calcium oxalate crystal growth was thought to decrease because of a reduction in urinary citrate and pH (Fleisch, 1978; Sheehan & Nancollas, 1981). Whether the urinary macromolecular inhibitory activity of crystal aggregation was affected cannot be determined for certain, but as uric acid crystals may adsorb urinary macromolecular inhibitors in the presence of calcium and oxalate in solution (III) such an effect may well have occurred since uric acid was demonstrably supersaturated on HPD.

GENERAL SUMMARY

1. Hyperuricaemia or hyperuricosuria was no more common in calcium stone formers than in healthy controls.

2. Patients who had hyperuricaemia or hyperuricosuria had a complicated stone disease with a high frequency of stone operations.

3. Patients with renal acidification defects in combination with hyperuricaemia or hyperuricosuria had the highest stone activities and stone operation rates, whereas patients with only renal acidification defects were on a par with stone patients with no manifest metabolic abnormalities.

4. Hyperuricosuria in calcium stone patients was not explained by a renal tubular dysfunction of the urate transport.

5. Proximal renal tubular acidification defect was associated with a high serum urate, a low urate clearance and a high fractional reabsorption of filtered urate.

6. Calcium oxalate crystals adsorbed almost all the macromolecular inhibitors but only a small fraction of the ultrafiltrable inhibitors, indicating a more permanent adsorption of macromolecular inhibitors on these crystals.

7. Monosodium urate or uric acid crystals barely adsorbed urinary macromolecular inhibitors of crystal growth, but in the presence of calcium and oxalate in the solution both crystal types seemed to adsorb a significant amount of the inhibitors.

8. A high content of animal protein in the diet caused an increase in the urinary excretion of calcium and a decrease in urinary pH and the excretion of citrate, a pattern which is thought to be unfavourable for renal calcium stone formation.

9. The increase in urinary calcium on high protein diet was interpreted as being a primary renal loss of calcium.

10. The changes in urinary pH and urinary urate caused a ten-fold increase in the ion activity of uric acid. All urines became supersaturated with uric acid on the high protein diet.

In conclusion it is believed that disturbances in urate metabolism are not a common characteristic of renal calcium stone patients but when present are associated with a more complicated course of the disease, probably including an interaction with urinary inhibitors of crystal growth and aggregation. The animal protein content of the diet could be of importance for such a development of calcium urolithiasis.

ACKNOWLEDGEMENTS

The present investigation was carried out at the Departments of Internal Medicine and Geriatrics, University Hospital, and the Department of Physiology, Biomedical Centre, University of Uppsala, Sweden.

I wish to express my gratitude to:

HARRY BOSTRÖM, the head of the Department of Internal Medicine, for providing excellent working conditions and for his interest in my work.

BO G. DANIELSON and SVERKER LJUNGHALL, for introducing me to urolithiasis research, for encouraging me to do these studies and for an enthusiastic and never failing support during the course of the work.

BJÖRN WIKSTRÖM, for his co-authorship and his patience in sharing an office with me in the clinic during this period.

ULLA BACKMAN, GUNNAR JOHANSSON and KLAS HOLMGREN, for a stimulating co-authorship and fine companionship.

BRITA KARLSTRÖM, HANS LITHELL and BENGT VESSBY, for a fine collaboration and for introducing me to nutritional research.

LEIF WIDE, KRISTER HELLSING and BERTIL KARLMARK, for inspiring discussions on chemical analysis and renal physiology.

KARL-ERIK FJELLSTRÖM, my teacher in nephrology, for his interest in the progress of this work.

ELISABETH PALLIN, AGNETA WÄRNGREN and GULL LUNDIN, for extensive help in the collection of data, and accuracy in the laboratory work.

ELISABET OLSSON and CHRISTL RICHTER, for their patience in the repeated typing of all the manuscripts and drawing of all the figures.

JEAN GRAY, for revising my own crude translation to the English language.

MY COLLEAGUES, for referring the patients and for stimulating discussions about the current topic.

MY FAMILY, for providing many of the basic requirements during this period.

This work was supported by the Swedish Medical Research Council, Project No. 2329, and the Åke Wiberg Foundation.

REFERENCES

Allen, L.H., Bartlett, R.S. & Block, G.D., 1979. Reduction of renal calcium reabsorption in man by consumption of dietary protein. J Nutr 109, 1345-1350.

Andersen, D.A., 1969. Historical and geographical differences in the pattern of incidence of urinary stones considered in relation to possible aetiological factors. In Renal Stone Research Symposium (Eds. A. Hodgkinson, B.E.C. Nordin), p. 7-32, Churchill, London.

Andersson, H., 1978. Hyperoxaluria in malabsorptive states. In Oxalate in Human Biochemistry and Clinical Pathology (Eds G.A. Rose, W.G. Robertson, R.W.E. Watts), p. 231-234, Proceedings of an International Symposium, Wellcome Foundation, London.

Arnaud, C.D., Tsao, H.S. & Littledike, T., 1971. Radioimmunoassay of human parathyroid hormone in serum. J Clin Invest 50, 21-34.

Arnold, W.J., 1978. Purine salvage enzymes. In: Uric acid. (Eds W.N. Kelley, I.M. Weiner), pp 43-73. Springer Verlag, Berlin.

Ayvazian, J.H. & Ayvazian, L.F., 1961. A study of hyperuricemia induced by hydrochloro-thiazide and acetazolamide separately and in combination. J Clin Invest 40, 1961-1966.

Backman, U., Danielson, B.G., Johansson, G., Ljunghall, S. & Wikström, B., 1979. Effects of therapy with bendroflumethiazide in patients with recurrent renal calcium stones. Br J Urol 51, 175-180.

Backman, U., Danielson, B.G., Johansson, G., Ljunghall, S. & Wikström, B., 1980a. Incidence and clinical importance of renal tubular defects in recurrent renal stone formers. Nephron 25, 96-101.

Backman, U., Danielson, B.G., Fellström, B., Johansson, G., Ljunghall, S. & Wikström, B., 1980b. Kidney stone disease. Experiences from Uppsala, Sweden. Scand J Urol Nephrol 53 (suppl.), 207-211.

Barlow, K.A. & Beilin, L.J., 1968. Renal disease in primary gout. Quart J Med 145, 79-96.

Barzel ,U.S., Sperling, O., Frank, M. & de Vries, A., 1964. Renal ammonium excretion and urinary pH in idiopathic uric acid lithiasis. J Urol 92, 1-5.

Batuman, V., Maesaka, J.K., Haddad, B., Tepper, E., Landy, E. & Wedeen, R.P., 1981. The role of lead in gout nephropathy. N Engl J Med 304, 520-523.

Beck, L.H., 1979. Hypouricemia in the syndrome of inappropriate secretion of antidiuretic hormone. N Eng J Med 301, 528-530.

Blomstedt, J.W. & Aronson, P.S., 1980. pH-gradient-stimulated transport of urate and p-amino-hippurate in dog renal microvillus membrane vesicles. J Clin Invest 65, 931-934.

Boss, G.R. & Seegmiller, J.E., 1979. Hyperuricemia and gout. N Engl J Med 300, 1459-1468.

Bowyer, R.C., Brockis, J.G. & McCulloch, R.K., 1979. Glycosaminoglycans as inhibitors of calcium oxalate crystal growth and aggregation. Clin Chim Acta 95, 23-28.

Brand, E., Harris, M.M. & Biloon, S., 1930. Cystinuria: The excretion of a cystine complex which decomposes in the urine with the liberation of free cystine. J Biol Chem 86, 315-331.

Butz, M., Hoffman, H., Kohlbecker, G. & Brosig, W., 1981. The influence of dietary protein on serum and urinary oxalate. In Urolithiasis: Clinical and basic research (Ed. L.H. Smith). In press, Plenum Publishing Company, New York.

Cannon, P.J., Stason, W.B., Demartini, F.E., Sommers, S.C. & Laragh, J.H., 1966. Hyperuricemia in primary and renal hypertension. N Engl J Med 275, 457-464.

Cifuentes-Delatte, L., Rapado, A., Abehsera, A., Traba, M.L. & Cortes, M., 1973. Uric acid lithiasis and gout. In Urinary Calculi Int. Symposium Renal Stone Research (Eds. L. Cifuentes-Delatte, A. Rapado, A. Hodgkinson), p. 115-118, Karger, Basel.

Coe, F.L., 1977. Treated and untreated recurrent calcium nephrolithiasis in patients with idiopathic hypercalciuria, hyperuricosuria or no metabolic disorders. Ann Int Med 87, 404-410.

Coe, F.L. & Kavalach, A.G., 1974. Hypercalciuria and hyperuricosuria in patients with calcium nephrolithiasis. N Engl J Med 291, 1344-1350.

Coe, F.L., Lawton, R.L., Goldstein, R.B. & Tembe, V., 1975. Sodium urate accelerates precipitation of calcium oxalate in vitro. Proc Soc Exp Biol Med 149, 926-929.

Coe, F.L., Moran, E. & Kavalach, A.G., 1976. The contribution of dietary purine overconsumption to hyperuricosuria in calcium oxalate stone formers. J Chron Dis 29, 793-800.

Cohen, J.J. & Barac-Nieto, M., 1973. Renal metabolism of citrate. In: Handbook of Physiology. Renal Physiology (Eds. J. Orloff, R.W. Berliner) American Physiological Society, pp 948-961, Williams & Wilkins, Baltimore.

Colton, R.S., Ward, L.E., Maher, F.T. & Ferguson, R.H., 1966. Occult renal impairment in gouty and hyperuricemic individuals. Am J Med Sci 252, 575-579.

Comper, W.D. & Laurent, T.C., 1978. Physiological function of connective tissue polysaccharides. Physiological Reviews 58, No 1, 255-315.

Danielson, B.G., Johansson, G. & Ljunghall, S., 1979. Magnesium metabolism in healthy subjects. Scand J Urol Nephrol 51, (suppl) 49-73.

Dent, C.D. & Sutor, D.J., 1971. Presence or absence of inhibitor of calcium oxalate crystal growth in urine of normals and of stone-formers. Lancet i(i), 775-778.

Diamond, H.S. & Paolino, J.S., 1973. Evidence for a postsecretory reabsorptive site for uric acid in man. J Clin Invest 52, 1491-1499.

Emmerson, B.T., 1967. Metals and the kidney. In: Renal disease. (Ed. D.A.K. Black), p 575., Blackwell, Oxford.

Emmerson, B.T., 1978. Abnormal urate excretion associated with renal and systemic disorders, drugs and toxins. In: Uric acid. (Eds. W.N. Kelley, I.M. Weiner), pp 287-324. Springer Verlag, Berlin.

Engle, J.E. & Steele, T.H., 1976. Variation of urate with urine flow in normal man. Nephron 16, 50-56.

Ferris, T.F. & Gorden, P., 1968. Effect of angiotensin and norepinephrine upon urate clearance in man. Am J Med 44, 359-365.

Fessel, W.J., 1979. Renal outcomes of gout and hyperuricemia. Am J Med 67, 74-82.

Fessel, W.J., Siegelaub, A.B. & Johnson, E.S., 1973. Correlates and consequences of asymptomatic hyperuricemia. Arch Intern Med 132, 44-54.

Finlayson, B., 1977. Calcium stones; some physical and clinical aspects. In: Calcium Metabolism in Renal Failure and Nephrolithiasis (Ed. D.S. David), pp 337-382, John Wiley & Sons, New York.

Finlayson, B. & DuBois, L., 1978. Adsorption of heparin on sodium acid urate. Clin Chim Acta 84, 203-206.

Fleisch, H., 1978. Inhibitors and promoters of stone formation. Kidney Int 13, 361-371.

Frank, M., Many, M. & Sperling, O., 1979. Familial renal hypouricaemia: Two additional cases with uric acid lithiasis. Br J Urol 51, 88-91.

Fujiwara, Y., Takamitsu, Y., Ueda, N., Orita Y, & Abe, H., 1980. Hypouricemia due to an isolated defect in renal tubular urate reabsorption. Clin Nephrol 13, 44-48.

Gibson, T., Hannan, S.F., Hatfield, P.J., Simmonds, H.A.; Cameron, J.S., Potter, C.S. & Crute, C.M., 1977. The effect of acid loading on renal excretion of uric acid and ammonium in gout. Adv Exp Med Biol 76B, 46-55.

Goldfinger, S., Klinenberg, J.R. & Seegmiller, J.E., 1965. Renal retention of uric acid induced by infusion of beta-hydroxybutyrate and acetoacetate. N Engl J Med 272, 351-355.

Gonick, H.C., Rubini, M.E., Gleason, I.O. & Sommers, S.C., 1965. The renal lesion in gout. Ann Int Med 62, 667-674.

Gorden, P., Robertson, G.L. & Seegmiller, J.E., 1971. Hyperuricemia, a concomitant of congenital vasopressin-resistant diabetes insipidus in the adult. N Engl J Med 284, 1057-1060.

Gutman, A.B. & Yü, T.-F., 1963. An abnormality of glutamine metabolism in primary gout. Am J Med 35, 820-831.

Gutman, A.B. & Yü, T.-F., 1965. Urinary ammonium excretion in primary gout. J Clin Invest 44, 1474-1481.

Gutman, A.B. & Yü, T.-F., 1968. Uric acid nephrolithiasis. Am J Med 45, 756-779.

Hehrmann, R., Nordmeyer, J.P., Wilke, R. & Hesch, R.-D., 1977. Radioimmunoassay of human PTH: characterization of two new antisera from sheep and clincal results. Acta Endocrinol 208, (suppl) 117-118.

Hering, F., Bigalke, K.-H. & Lutzeyer, W., 1979. Interaction of hyperuricuria and hyperoxaluria on renal calcium oxalate stone formation. In: Purine Metabolism in Man III (Eds. A. Rapado, R.W.E. Watts, C.H.M.M. de Bruyn), Vol 22A, pp 99-107. Plenum Press, New York.

Hitchings, G.H., 1978. Uric acid chemistry and synthesis. In: Uric acid (Eds. W.N. Kelley, I.M. Weiner), pp 1-20. Springer Verlag, Berlin.

Hodgkinson, A., 1976. Uric acid disorders in patients with calcium stones. Br J Urol 48, 1-5.

Hodgkinson, A. & Williams, A., 1972. An improved colorimetric procedure for urine oxalate. Clin Chim Acta 36, 127-132.

Holmes, E.W., 1978. Regulation of biosynthesis de novo. In: Uric acid. (Eds. W.N. Kelley, I.M. Weiner), pp 21-41. Springer Verlag, Berlin.

Johansson, G., 1979. Magnesium metabolism. Studies in health, primary hyperparathyroidism and renal stone disease. Scand J Urol Nephrol 51, (suppl) 1-47.

Johansson, G., Backman, U., Danielson, B.G., Fellström, B., Ljunghall, S. & Wikström, B., 1980. Biochemical and clinical effects of the prophylactic treatment of renal calcium stones with magnesium hydroxide. J Urol 124, 770-774.

Johnson, N.E., Alcantara, E.N. & Linksweiler, H., 1970. Effect of level of protein intake on urinary and fecal calcium and calcium retention of young adult males. J Nutr 100, 1425-1430.

Kageyama, N., 1971. A direct colorimetric determination of uric acid in serum and urine with uricase-catalase system. Clin Chim Acta 31, 421-426.

Karlmark, B. 1980. CO_2 permeability of the nephron in relation to the nature of luminal fluid acidification. Ann N Y Acad Sci 341, 111-116.

Katz, W.A., 1978. Role of proteoglycans in the development of gouty arthritis. In: Uric acid. (Eds. W.N. Kelley, I.M. Weiner), pp 347-364, Springer Verlag, Berlin.

Kjellstrand, C.M., Campbell, D.C., von Hartitzsch, B. & Buselmeier, T.J., 1974. Hyperuricemic acute renal failure. Arch Intern Med 133, 349-359.

Klinenberg, J.R., Dornfield, L.P. & Gonick, H.C., 1967. Renal function abnormalities in patients with asymptomatic hyperuricemia. Arthritis Rheum 12, 307.

Klinenberg, J.R., Kippen, I. & Bluestone, R., 1975. Hyperuricemic nephropathy: Pathologic features and factors influencing urate deposition. Nephron 14, 88-98.

Lang, F., Greger, R., Oberleithner, H., Griss, E., Lang, K., Pastner, D., Dittrich, P. & Deetjen, P., 1980. Renal handling of urate in healthy man in hyperuricaemia and renal insufficiency: circadian fluctuation, effect of water diuresis and of uricosuric agents. Eur J Clin Invest 10, 285-292.

Lang, F., Quehenberger, P., Greger, R. & Oberleitner, H., 1978. Effect of benzolamide on luminal pH in proximal convoluted tubules of the rat kidney. Pflüger Arch 375, 39-43.

Lemann, J., Piering, W.F. & Lennon, E.J., 1969. Possible role of carbohydrate-induced calciuria in calcium oxalate kidney stone formation. N Engl J Med 280, 232-237.

Lemieux, G., Ninay, P., Gougoux, A. & Michaud, G., 1973. Nature of the uricosuric action of benziodarone. Am J Physiol 224, 1440-1449.

Lennon, E.J., Lemann, J. & Relman, A.S., 1962. The effects of phosphoproteins on acid balance in normal subjects. J Clin Invest 41, 637-645.

Ljunghall, S., 1977. Renal stone disease. Studies of epidemiology and calcium metabolism. Scand J Urol Nephrol 41, (suppl) 6-38.

Ljunghall, S., Backman, U., Danielson, B.G., Johansson, G., Jung, B., Källsen, R., Wibell, L. & Wålinder, O., 1977. Mechanisms of hypercalciuria in recurrent renal stone formers. Scand J Urol Nephrol 41, (suppl) 77-96.

Ljunghall, S., Backman, U., Danielson, B.G., Fellström, B., Johansson, G. & Wikström, B., 1980. Calcium metabolism in renal stone disease. Scand J Urol Nephrol 53 (suppl.), 111-120.

Ljunghall, S., Backman, U., Danielson, B.G., Fellström, B., Johansson, G., Odlind, B. & Wikström, B., 1981. Effects of thiazides on urate metabolism during long-term treatment of patients with renal stones. In manuscript.

Lonsdale, K., 1968. Human stones. Science 159, 1199-1207.

Löffler, W., Gröbner, W. & Zöllner, N., 1979. Influence of dietary protein on serum and urinary uric acid. In: Purine Metabolism in Man III. (Eds. A. Rapado, R.W.E. Watts, C.H.M.M. de Bruyn), Vol 122A, pp 209-214. Plenum Press, New York.

Manuel, M.A. & Steele, T.H., 1974. Changes in renal urate handling after prolonged thiazide treatment. Am J Med 57, 741-746.

Marshall, R.W. & Robertson, W.G., 1976. Nomograms for the estimation of the saturation of urine with calcium oxalate, calcium phosphate, magnesium ammonium phosphate, uric acid, sodium acid urate, ammonium acid urate and cystine. Clin Chim Acta 72, 253-260.

Matzkies, F., Berg, G. & Mädl, H., 1979. The uricosuric action of protein in man. In: Purine Metabolism in Man III (Eds. A. Rapado, R.W.E. Watts, C.H.M.M. de Bruyn), Vol 122A, pp 227-232. Plenum Press, New York.

McCance, R.A., Widdowson, E.M. & Lehmann, H., 1942. The effect of protein intake on the absorption of calcium and magnesium. Biochem 36, 686-691.

Meisel, A. & Diamond, H., 1976. Effect of vasopressin on uric acid excretion: evidence for distal nephron reabsorption of urate in man. Clin Sci Mol Med 51, 33-40.

Messerli, F.H., Frohlich, E.D., Dreslinski, G.R., Suarez, D.H. & Aristimuno, G.G., 1980. Serum uric acid in essential hypertension: An indicator of renal vascular involvement. Ann Int Med 93, 817-821.

Metcalfe-Gibson, A., McCallum, F.M., Morrison, R.B.I. & Wrong, O., 1965. Urinary excretion of hydrogen ion in patients with uric acid calculi. Clin Sci 28, 325-342.

Morris, R.C., Sebastian, A. & MacSherry, E., 1972. Renal adicosis. Kidney Int 1, 322-340.

Padova, J. & Bendersky, G., 1962. Hyperuricemia in diabetic keto-acidosis. N Engl J Med 267, 530-534.

Pak, C.Y.C. & Arnold, L.H., 1975. Heterogenous nucleation of calcium oxalate by seeds of monosodium urate. Proc Soc Exp Biol Med 149, 930-932.

Pak, C.Y.C., Barilla, D., Holt, K., Brinkley, L., Tolentino, R. & Zerwekh, J., 1978. Effect of oral purine load and allopurinol on the crystalization of calcium salts in urine of patients with hyper-uricosuric calcium urolithiasis. Am J Med 65, 593-599.

Pak, C.Y.C., Holt, K. & Zerwekh, J.E., 1979. Attenuation by mono-sodium urate of the inhibitory effect of glycosaminoglycans on calcium oxalate nucleation. Invest Urol 17, 138-141.

Pak, C.Y.C., Ohata, M., Lawrence, E.C. & Snyder, W., 1974. The hypercalciurias: Causes, parathyroid functions and diagnostic criteria. J Clin Invest 54, 387-400.

Pak, C.Y.C., Waters, O., Arnold, L.R., Holt, K., Cox, C. & Barilla, D., 1977. Mechanism for calcium urolithiasis among patients with hyperuricosuria. J Clin Invest 58, 126-131.

Pak Poy, R.K., 1965. Urinary pH in gout. Aust Ann Med 4, 35-39.

Pollak, V.E. & Mattenheimer, H., 1965. Glutaminase activity in the kidney in gout. J Lab Clin Med 66, 564-570.

Rapado, A., Castrillo, J.M., Diaz-Curiel, M., Traba, M.L., Santos, M. & Cifuentes-Delatte, L., 1979. Uric acid/calcium oxalate nephrolithiasis. Clinical and biochemical findings in 86 patients. In: Purine Metabolism in Man III. (Eds. A. Rapado, R.W.E. Watts, C.H.M.M. de Bruyn), Vol 122A, pp 109-114. Plenum Press, New York.

Rich, W., 1977. Ion chromatography: A new technique for the automated analysis of ions in solutions. Analysis Instrumentation 15.

Rieselbach, R.E., Sørensen, L.B., Shelp, W.D. & Steele, T.H., 1970. Diminished renal urate secretion per nephron as a basis for primary gout. Ann Int Med 73, 359-366.

Robertson, W.G., 1981. Studies on the risk of urinary stone formation in vegetarians. (Abstract). In press, Harnstein-Symposium, Bonn-Wien.

Robertson, W.G., Heyburn, P.J., Peacock, M., Hanes, F.A. & Swaminathan, R., 1979a. The effects of high animal protein intake on the risk of calcium stone formation in the urinary tract. Clin Sci 57, 285-288.

Robertson, W.G., Knowles, F. & Peacock, M., 1976a. Urinary acid mucopolysaccharide inhibitors of calcium oxalate crystallisation. In: Urolithiasis Research (Eds. H. Fleisch, W.G. Robertson, L.H. Smith, W. Vahlensieck), pp 331-334. Plenum Press, New York.

Robertson, W.G. & Peacock, M., 1980. The cause of idiopathic calcium stone disease: Hypercalciuria or hyperoxaluria? Nephron 26, 105-110.

Robertson, W.G., Peacock, M., Heyburn, P.J., Hanes, F.A., Rutherford, A., Clementson, E., Swaminathan, R. & Clark, P.B., 1979b. Should recurrent calcium oxalate stone formers become vegetarians? Br J Urol 51, 427-431.

Robertson, W.G., Peacock, M., Heyburn, P.J., Marshall, D.H. & Clark, P.B., 1978a. Risk factors in calcium stone disease of the urinary tract. Br J Urol 50, 449-454.

Robertson, W.G., Peacock, M., Heyburn, P.J., Speed, R. & Hanes, F., 1978b. The role of affluence and diet in the genesis of calcium containing stones. In Pathogenese und Klinik der Harnstein VI, pp. 5-14, Steinkopff, Darmstadt.

Robertson, W.G., Peacock, M., Marshall, R.W., Marshall, D.H. & Nordin, B.E.C., 1976b. Saturation-inhibition index as a measure of the risk of calcium oxalate stone formation in the urinary tract. N Engl Med J 294, 249-252.

Robertson, W.G., Peacock, M. & Nordin, B.E.C., 1968. Activity products in stone-forming and non-stone-forming urine. Clin Sci 34, 579-594.

Robertson, W.G., Peacock, M., Nordin, B.E.C., 1971. Calcium oxalate crystalluria and urine saturation in recurrent renal stone-formers. Clin Sci 40, 365-374.

Robertson, W,G,, Peacock, M. & Nordin, B.E.C., 1973. Inhibitors of the growth and aggregation of calcium oxalate crystals in vitro. Clin Chim Acta 43, 31-37.

Schrier, E.E., Lee, K.E., Rubin, J.L., Werness, P.G. & Smith, L.H., 1978. Macromolecular inhibitors of calcium oxalate crystal growth and aggregation in the urine. In: Oxalate in Human Biochemistry and Clinical Pathology (Eds. G.A. Rose, W.G. Robertson, R.W.E. Watts), pp 22-27. Proceedings of an International Symposium, Wellcome Foundation, London.

Schwille, P., Samberger, N. & Wach, B., 1976. Fasting uric acid and phosphate in urines and plasma of renal calcium stone formers. Nephron 16, 116-125.

Sheehan, M.E. & Nancollas, G.H., 1981. A constant composition method for modelling urinary stone formation. In Urolithiasis. Clinical and basic research. (Ed. L.H. Smith). In press, Plenum Publishing Company, New York.

Simmonds, H.A., van Acker, K.J., Dillon, M.J., Barratt, T.M., Potter, C.F,, Sahota, A. & Cameron, J.S., 1981. 2,8-dihydroxyadeninuria: or when is a uric acid stone not a uric acid stone? In: Urolithiasis: Clinical and basic research. (Ed. L.H. Smith). In press, Plenum Publishing Company, New York.

Smith, L.H., van den Berg, C.J. & Wilson, D.M., 1978. Current concepts in nutrition - Nutrition and Urolithiasis. N Engl J Med 298, 87-89.

Smith, M.J.V., Hunt, L.D., Cling, J.S. & Boyce, W.H., 1969. Uricemia and urolithiasis. J Urol 101, 637-642.

Sohtell, M., 1979. CO_2 along the proximal tubules in the rat kidney. Acta Phys Scand 105, 146-155.

Sørensen, L.B. & Levinson, D.J., 1975. Origin and extrarenal elimination of uric acid in man. Nephron 14, 2-20.

Sørensen, L.B. & Levinson, D.J., 1976a. Clinical evaluation of benzbromarone. A new uricosuric drug. Arthritis Rheum 19, 183-190.

Sørensen, L.B. & Levinson, D.J., 1976b. Evidence for four compartments in the renal handling of uric acid in man. In: Amino Acid Transport and Uric Acid Transport. (Eds. S. Silbernagl, F. Lang, R. Greger), pp 243-247. Georg Thieme Publishers, Stuttgart.

Spann, W.K., Gröbner, W. & Zöllner, N., 1979. Effect of hypoxanthine in meat on serum uric acid and urinary uric acid excretion. In: Purine Metabolism in Man III. (Eds. A. Rapado, R.W.E. Watts, C.H.M.M. de Bruyn), Vol 122A, pp 215-220. Plenum Press, New York.

Steele, T.H., 1969. Evidence for altered renal urate reabsorption during changes in volume of the extracellular fluid. J Lab Clin Med 74, 288-299.

Steele, T.H., 1973. Urate secretion in man: The pyrazinamide suppression test. Ann Int Med 79, 734-737.

Steele, T.H., 1978. Urate excretion in man, normal and gouty. In: Uric acid, (Eds. W.N. Kelley & I.M. Weiner), pp 257-286. Springer Verlag, Berlin.

Steele, T.H, Manuel, M.A. & Boner, G., 1975. Diuretics, urate excretion and sodium reabsorption - effects of acetazolamide and urinary alkalinization. Nephron 14, 48.

Steele, T.H. & Oppenheimer, S., 1969. Factors affecting urate excretion following diuretic administration in man. Am J Med 47, 564-

Steele, T.H. & Rieselbach, R.E., 1967. The contribution of residual nephrons within the chronically diseased kidney to urate homeostasis in man. Am J Med 43, 876-886.

Talbott, J.H., 1978. Pathology of urate nephropathy. In: Uric acid. (Eds. W.N. Kelley, I.M. Weiner), pp 443-458. Springer Verlag, Berlin.

Talbott, J.H. & Terplan, K.L., 1960. The kidney in gout. Medicine 39, 405-462.

Thom, J.A., Morris, J.E., Bishop, A. & Blacklock, N.J., 1978. The influence of refined carbohydrate on urinary calcium excretion. Br J Urol 50, 459-464.

Varadi, D.P., Cifonelli, J.A. & Dorfman, A., 1967. The acid mucopolysaccharides in normal urine. Biochem Biophys Acta 141, 103-117.

Vieira, F.L. & Malnic, G., 1968. Hydrogen ion secretion by rat renal cortical tubules as studied by antimony microelectrode. Am J Physiol 214, 710-718.

de Vries, A., 1980. Purine metabolism in uric acid lithiasis. Scand J Urol Nephrol 53, (suppl) 161-168.

de Vries, A., Weinberger, A. & Frank, M., 1973. Treatment of uric acid lithiasis: observations on 658 patients. In Urinary Calculi. Int. Symposium Renal Stone Research (Eds. L. Cifuentes-Delatte, A. Rapado, A. Hodgkinson), p. 105-110, Karger, Basel.

Weiner, I.M. & Fanelli, G.M., 1975. Renal urate excretion in animal models. Nephron 14, 33-47.

Welshman, S.G. & McCambridge, H., 1973. The estimation of citrate in serum and urine using a citrate lyase technique. Clin Chim Acta 46, 243.

Wessler, E., 1971. The nature of the non-ultrafilterable glycosaminoglycans of normal human urine. Biochem J 122, 373-384.

Wikström, B., Backman, U., Danielson, B.G., Fellström, B., Johansson, G. & Ljunghall, S., 1981. Urinary citrate excretion and acidification defects in renal calcium stone formers. In: Urolithiasis: Clinical and basic research. (Ed. L.H. Smith). In press, Plenum Publishing Company, New York.

Williams, H.E., 1976. Oxalic acid: Absorption, excretion and metabolism. In: Urolithiasis Research (Eds. H. Fleisch, W.G. Robertson, L.H. Smith, W. Vahlensieck), pp 181-188. Plenum Press, New York.

Wilson, D.M. & Goldstein, N.P., 1973. Renal urate excretion in patients with Wilson's disease. Kidney Int 4, 331-336.

Wålinder, O., Ljunghall, S. & Wibell, L., 1978. Urinary excretion of cyclic AMP in hyperparathyroidism. Scand J Urol Nephrol 12, 67-71.

Yü, T.-F. & Gutman, A.B., 1955. Paradoxical retention of uric acid by uricosuric drugs in low dosage. Proc Soc Exp Biol 90, 542-

Yü, T.-F. & Gutman, A.B., 1967. Uric acid nephrolithiasis in gout. Predisposing factors. Ann Int Med 67, 1133-1148.

Zöllner, N., 1976. Influence of diet on urinary uric acid excretion. In: Urolithiasis Research (Eds. H. Fleisch, W.G. Robertson, L.H. Smith, W. Vahlensieck), pp 155-163. Plenum Press, New York.

Printed in Sweden by
Centraltryckeriet AB Uppsala, 1981